

THE INFLUENCE AND THE ROLE OF POLYPHENOLS ON THE SENSORY AND ORGANOLEPTIC ASPECTS OF FOOD

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Summary

A vast majority of food products found on the market contains specific, potentially toxic substances, whether those substances appeared as a result of food contamination by an exterior factor or during the production. As an examples of food contamination we can consider bacterial and fungal toxins, where the latter (mycotoxins) include some evidently mutagenic or genotoxic compounds, i.e. potentially cancerogenic compounds. Mycotoxins may enter the food chain via direct or indirect contamination. In direct contamination, the food products represent the basis for the development of toxigenic moulds (almost all food products may represent a basis for the mould development during their production, processing and storage). Bioactive plant polyphenols are relatively well-known for their antioxidant, anti-mutagenic, anticancerogenous, anti-inflammatory, antiangiogenic, antiulcer and antimicrobial characteristics. Many plant ingredients and extracts are reported to prevent the mould development, as well as the accumulation of mycotoxins in food. In addition to their multiple biological effects, extracts containing a large amount of phenols are important for the food industry, since they decelerate the oxidative degradation of lipids, thus enhancing the quality and nutritive value of food. On the other hand, plant polyphenols affect the sensory and organoleptic aspects of food, the functional and nutritive value of the proteins contained in food, as well as its texture.

Keywords: antioxidant activity, *Echinacea purpurea*, polyphenols, sensory analysis

Introduction

Mycotoxin contamination of food for human use, as well as of food for animal use, is a public health problem. Limits of contamination of specific foods are defined by the laws of the state governments (Tritscher, 2004; Kumar et al., 2008). Mycotoxins are low molecular metabolites which are formed by certain strains of different mold species from *Aspergillus*, *Penicillium*, *Fusarium* and other genera. They can enter the food chain directly or indirectly by food contamination as a substrate. In direct contamination, the food material alone is a basis of toxigenic mold growth. Almost all foods can be a substrate for the growth of mold during their production, processing, transportation and storage. In contrast, indirect contamination will occur if food supplements are contaminated with mycotoxins. The real danger of mycotoxins is reflected in their delayed activity because they can cause a variety of mutagenic and carcinogenic changes at the cellular level (Knasmüller et al., 2001; Clark et al., 2006). At the same time, it is impossible to avoid their presence in foods, and thus exposure. Species of *Echinacea* L. genus are scientifically established immunomodulators and phytochemical composition indicates the presence of substances with antioxidant and anti-inflammatory effects (Barnes et al., 2005; Kosalec, 2006).

Materials and methods

Plant material

This research used air dried aerial parts of purple *Echinacea*, taken from the Jan-Spider (Pitomača, HR). In order to analyse the potential use of plants extract in the treatment of food products (peanuts and raisins), including sensory analysis, samples of peanuts were used from EuroCompany 99 (Ljubuški, BiH), as well as raisins from Bernina (Široki Brijeg, BiH).

Chemicals

Acetic acid, aluminium chloride, ethanol, ethylenediaminetetraacetic acid (EDTA), hexamethylenetetramine, methanol, pyrogallol, sodium carbonate, sodium citrate, sodium hydroxide, sodium nitrite, tannic acid (95%), thiourea were purchased from Kemika (Zagreb, Croatia). Chlorogenic acid, 2,2-diphenyl-1-picryl-hydrazyl (DPPH[•]), hydrogen peroxide, potassium ferricyanide, rosmarinic acid (96%), 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), dimethylsulfoxide (DMSO) and sodium molybdate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Butylated hydroxytoluene (BHT, ≥99%) and quercetin-3-

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rutinoside (rutin, $\geq 95\%$) were obtained from Fluka (Buchs, Switzerland). Folin–Ciocalteu's phenol reagent, 3-*tert*-butyl-4-hydroxyanisole (BHA) and were obtained from Merck (Darmstadt, Germany). Hydrochloric acid were obtained from Riedel-de Haën (Seelze, Germany) respectively. All chemicals and reagents used were of the highest analytical grade and obtained from "Kemika" Zagreb (Croatia).

Determination of total polyphenols, tannins, flavonoids and phenolic acids

Determination of total tannin as well as total polyphenol contents was performed following the method described in European Pharmacopoeia (EDQM, 2004). The percentage content of tannins, expressed as pyrogallol, was calculated from the following equation: $(\%) = 3.125 \times (A_1 - A_2) / (A_3 \times m)$, where A_3 is the absorbance of the test solution containing 0.05 g of pyrogallol, and m the mass of the extract (g).

The total flavonoid contents of tested plant extract were determined using the spectrophotometric method of Christ et al., (1960). All determinations were performed in triplicate. The percentage content of flavonoids, expressed as quercetin, was calculated from the equation: $(\%) = A \times 0.772 / b$, where A is the absorbance of the test solution at 425 nm and b is the mass of the sample, in grams.

Determination of hydroxycinnamic acid derivatives was performed according to procedure described in European Pharmacopoeia (EDQM, 2004). The percent of total hydroxycinnamic acid content was calculated and expressed as rosmarinic acid, according to the following expression: $(\%) = A \times 5.3 / m$, where A is the absorbance of the test solution at 525 nm and m is the mass of the sample, in grams. Analysis of each sample was performed in triplicate.

2, 2-Diphenyl-1-picrylhydrazyl radical (DPPH[•]) radical scavenging assay

The free radical scavenging activities of the samples were measured using the stable DPPH[•] radical, according to the method of Blois (1958). Briefly, 0.1 mM solution of DPPH[•] in ethanol was prepared and 1 mL of this solution was added to 3 mL of sample solution in ethanol at different concentrations (0.39–200 $\mu\text{g/mL}$). The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm. The capability to scavenge the DPPH[•] radical was calculated using the following equation: $(\%) = [(A_0 - A_1) / A_0] \times 100$, where A_0 is the absorbance of the

control reaction and A_1 is the absorbance in the presence of sample, corrected for the absorbance of sample itself. Butylated hydroxytoluene (BHT) was used for comparison. All determinations were done in triplicate.

Hydroxyl radical scavenging activity

As hydroxyl free radicals ($\bullet\text{OH}$) are highly reactive, with relatively short half-lives, the concentrations found in natural systems are usually inadequate for direct detection by ESR spectroscopy. Spin-trapping is a chemical reaction that provides an approach to help overcome this problem. Hydroxyl radicals are identified because of their ability to form nitroxide adducts (stable free radicals form) from the commonly used DMPO as the spin trap (Buettnier, 1985). The Fenton reaction was conducted by mixing 200 μL of DMPO (112 mM), 200 μL of DMF, 200 μL of H_2O_2 (2 mM) and 200 μL of FeCl_2 (0.3 mM) (control). The influence of *E. purpurea* extract on the formation and stabilization of hydroxyl radicals was investigated by adding investigated extracts in the Fenton reaction system at the range of concentrations 0.025–1.5 mg/mL. ESR spectra were recorded after 5 minutes, with the following spectrometer settings: field modulation 100 kHz, modulation amplitude 0.226 G, receiver gain 5×10^5 , time constant 80.72 ms, conversion time 327.68 ms, center field 3,440.00 G, sweep width 100.00 G, x-band frequency 9.64 GHz, power 20 mW, temperature 23°C. The SA_{OH} value of the extract was defined as: $\text{SA}_{\text{OH}}^{\bullet} = 100 \times (h_0 - h_x) / h_0$ [%]; where h_0 and h_x are the height of the second peak in the ESR spectrum of DMPO-OH spin adduct of the control and the probe, respectively.

Superoxide anion radical scavenging activity

Superoxide anion radicals ($\text{O}_2^{\bullet-}$) were generated in the reaction system obtained by mixing 500 μL of dry dimethylsulfoxide (DMSO), 5 μL of KO_2 / crown ether (10 mM / 20 mM) prepared in dry DMSO and 5 μL of 2 M DMSO solution of DMPO as spin trap. The influence of extracts on the formation and transformation of superoxide anion radicals was obtained by adding the DMF solution of *E. purpurea* extract to the superoxide anion reaction system at the range of concentrations 0.005–0.1 mg/mL. After that the mixture was stirred for 2 min and transferred to a quartz flat cell ER-160FT. The ESR spectra were recorded on an EMX spectrometer from Bruker (Rheinstetten, Germany) under the following conditions: field modulation 100 kHz, modulation amplitude 4.00 G, receiver gain 1×10^4 , time constant 327.68 ms, conversion time 40.96 ms, center

field 3440.00 G, sweep width 100.00 G, x-band frequency 9.64 GHz, power 20 mW, temperature 23 °C. The $SAO_2^{\bullet-}$ value of the extract was defined as: $SAO_2^{\bullet-} = 100 \times (h_0 - h_x) / h_0$ [%]; where h_0 and h_x are the height of the second peak in the ESR spectrum of DMPO-OOH spin adduct of the control and the probe, respectively.

The application of extracts and sensory properties of treated food products

Polyphenol content and antioxidant effects in in-vitro conditions were the basis for the interpretation of mechanisms ability of enriching food with extracts rich in antioxidants and other phytochemicals, which in addition to a role in increasing sustainability of food products, can modulate the toxicity of mycotoxins present in the organism.

Selected concentration range is based on two main factors: 1-efficient concentration which has an antioxidant effect, 2-level which does not alter substantially the sensory properties of the treated products. The application of Echinacea plant extracts included spraying of lyophilized water extracts and drying them. Sensory analysis was performed with the methodology described by Riveros et al. (2009) for peanuts and the methodology used for raisins was described by Al-Farsi et al. (2005) The treatment of food (peanuts and raisins) was performed with optimized spraying of lyophilized plant water extract of *E. purpurea* in three concentrations (0.1; 0.5 i 1.0

mg/mL). After the spraying was performed, drying was carried out at room temperature during 24 hours. Upon drying was completed, sensory analyst conveyed the intensity of individual properties on the line specified property.

Data analysis

One-way analysis of variance (ANOVA) and multiple comparisons (Duncan's *post-hoc* test) were used to evaluate the significant difference of the data at $p < 0.05$.

Results and discussion

The total amount of polyphenols in Echinacea purpurea

The results of spectrophotometrical identification of the total amount of polyphenols (prepared as shown in chapter 2.2) are presented in the Table 1. The Table 1 shows the values of the measured absorptions of the specimens of the ethanol extract in *E. purpurea* and the estimated content of the total polyphenols, tannin, phenolic acid, flavonoids. It was determined that the overhead parts of the examined species contain ranging between 12.98 and 13.80% of polyphenols, tannin between 0.85 and 0.92%, 3.23 to 3.72% hydroxycinnamic derivatives and portions of flavonoid between 0.123 to 0.131%.

Table 1. Contents of phenolic acids, flavonoids, tannins and total polyphenols in *E. purpurea* extract

Plant extracts	Total polyphenols	Contens (%) Flavonoids	Phenolics acids	Tanins
<i>Echinacea purpurea</i>	13.31±0.43	0.126±0.004	3.47±0.25	0.863±0.003
Each value is the mean ± SD of three independent measurements				

Antioxidant activities of E. purpurea ethanolic extracts

Polyphenolic compounds such as flavonoids, phenolic acids and tannins are considered to be the major contributors to the antioxidant activity of medicinal plants, fruits and vegetables (Pereira et al., 2009; Rice-Evans et al., 1996). Therefore, in the present study five different assays were employed in order to determine and compare the antioxidant properties of selected *Echinacea* species, as well as to elucidate their mode of action. The antiradical activity of the ethanol extract of the overhead part of the species *Echinacea purpurea*, chlorogenic acid, rutin, tannic acid, in comparison to the synthetic

antioxidant butyl-hydroxy anisole (BHA). After measuring absorptions at 517, the percentage of the inhibition capacity of DPPH[•] radicals were calculated. The plant extract in lower amounts has quite a weaker effect than the synthetic antioxidant. Although it lags continually after the effect of BHA, the difference is significantly lowered in the amounts above 50 µg/ml. It was also revealed that the chlorogenic acid, rutin and tannic acid are better catchers of DPPH[•] than the referent antioxidant. The effect of BHA is equalised with the effect of rutin only at the amount of 12.5 µg/ml when it accomplished the inhibition above 85%.

The strongest antiradical activity was determined for the tannic acid which already in the amount of 0.78

$\mu\text{g/ml}$ accomplishes a 50% exhibition of DPPH^{\bullet} . The chlorogenic acid shows the same effect in the amount of $1.56 \mu\text{g/ml}$ and is equalised with the tannic acid in the concentration of $6.25 \mu\text{g/ml}$. In the figure 1 it is visible that the necessary concentrations are above $15 \mu\text{g/ml}$ in order to achieve a 50% inhibition. In the concentration higher than $50 \mu\text{g/ml}$ the effect of the extract approaches the effect of clear substances and BHA. The results show that flavonoids, phenolic acid and tannins, present in the examined species, equally contribute to the antiradical effect of the extract.

The research of Yokozawa et al. (1998) has shown that tannins and some flavonoids show an activity in relation to DPPH^{\bullet} radicals and that the activity is closely related to their chemical structure. With the increase in galic groups, the molecular mass and ortho-hydroxy groups in the structure, the activity of tanine increases, and the number and position of hydroxyl groups represent an important characteristic of flavonoids for "quenchers" free radicals. Fenglin et

al. (2004) released the results of the study of the 'scavengers' activity on DPPH^{\bullet} radicals of water-methanol extracts of more than 300 medicinal herbs. For 56 of the examined specimens they got EC_{50} values under $0,500 \text{ mg}$ of the specimen/ml of the extragent. The same authors attribute the activity of DPPH^{\bullet} radicals of plants to the present flavonoids and tannins in the extract. Chen et al. (2004) discovered that the chlorogenic acid most actively removes DPPH^{\bullet} radicals in plants, and that their activity in the same test is the same and larger than the activity of tocopherol. Orhan et al. (2009) got similar results when they studied antioxidant activities of the species *E. purpurea* and *E. pallida* by determining the catching capacity of DPPH^{\bullet} of free radicals and chelate ions of iron. A chloroform extract in air of dry plant material *E. purpurea* showed the greatest capacity of chelate iron ions (Orhan et al., 2009).

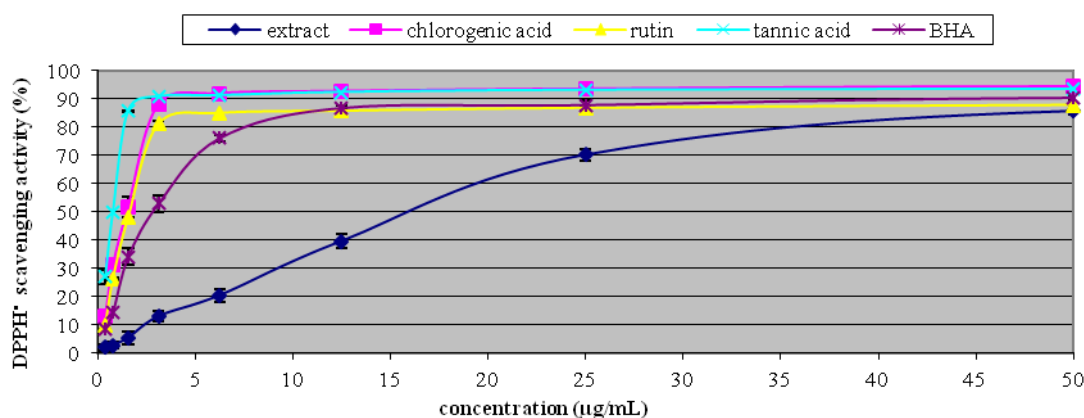


Fig. 1. DPPH free radical scavenging effects of *Echinacea* ethanolic extracts in comparison with polyphenolic compounds and a reference antioxidant

Results ESR

One part of our investigation on antioxidant activity of *E. purpurea* extract was the scavenging activities on hydroxyl and superoxide anion radicals measured by ESR method. Using a spin trap, such as DMPO, it is possible to convert reactive hydroxyl radicals to stable nitroxide radicals (DMPO-OH adducts) with spectral hyperfine splitting that reflects the nature and structure of these radicals. The reaction of Fe_2^{+} with H_2O_2 in the presence of the spin trapping agent DMPO generated a 1:2:2:1 quartet of lines with hyperfine coupling parameters ($a_N = a_H = 14.9 \text{ G}$) (Ćanadanović-Brunet, et al., 2005). The intensity of the ESR signal, corresponding to the concentration of free radicals formed, was decreased in the presence

of different amounts of *E. purpurea* extract. The total elimination of hydroxyl radical ($\text{SA}_{\text{OH}} = 100\%$) was obtained in the presence of 1.5 mg/ml of extract, which indicates that this applied concentration inhibits the creation of hydroxyl radicals completely. The investigated extract showed dose-dependent radical scavenging activities. The EC_{50} value, defined as the concentration of extract required for 50% scavenging of superoxide anion radicals under experimental condition employed, is a parameter widely used to measure the free radical scavenging activity (Cuvelier et al., 1992); a smaller EC_{50} value corresponds to a higher antioxidant activity. The EC_{50} value of *E. purpurea* extract (0.077 mg/ml) shows that extract is rich in antioxidant compounds and efficiently scavenge superoxide anion radicals.

Sensory properties of treated products

On the basis of the treatment of food products, by adding purple Echinacea extracts rich in antioxidants and other phytochemicals, quantitative polyphenol content, antioxidant activity of the extracts and the potential modulation of toxicity of mycotoxins present was determined. Efficient treatments would certainly be able to hold the sensory properties of foods within acceptable limits. During the testing, the samples of selected treated foods were evaluated and mean scores of individual sensory properties were calculated, as shown on the network diagrams (Fig. 2-3). In this way, by application of quantitative descriptive analysis, sensory profiles of foods treated with the tested plant extract were described. Table 2 shows that values of some sensory parameters are significantly different from control. According to the sensory profiles (Figure 3), the biggest deviations are visible at the highest concentration that could be taken as an optimal compromise between efficiency and impact on sensory properties.

The treatment of raisins with Echinacea extracts caused a statistically significant difference compared to the control sample for parameters of taste, flavor and aroma (Table 3). Also, according to Figure 4, the strongest deviation from the original product is that for parameter of smell per material/fruit. It is evident that the specific smell of plant extracts had masked the original smell of raisins. According to the comments of participants of the panel, this deficiency in relation to the average mean score of the control sample is not considered undesirable. Moreover, it resulted with attractive sensory profile of the treated samples. Paired comparison test clearly distinguished treated from untreated samples of raisins and peanuts. However, judging by the sensory profile, it can be concluded that the newly created formulations of

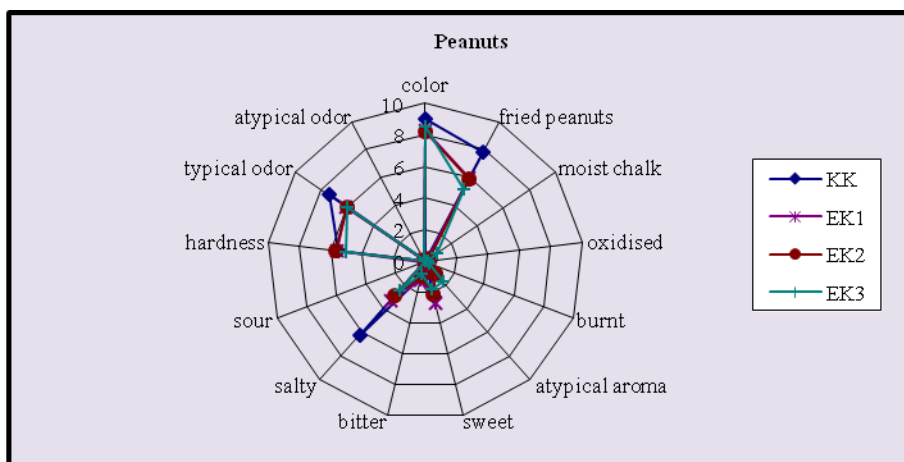
treated food products, with added extracts showed satisfactory sensory profile, with some exceptions. Finally, in the treatment of peanuts, attention should be kept regarding salinity or the choice of drying process during the treatment of salting. Taking into consideration the sensory evaluation of treated peanuts and raisins with Echinacea extracts, the plant would certainly have the ability to completely justify use in food industry. In doing so, it should be stressed that, according to the sensory profiles (Fig. 4), the optimal addition was the lowest concentration of tested extracts.

During the recent decades, many researchers and food manufacturers showed great interest for the natural phenolic compound. The main reason for this interest is their antioxidant activity, their representation in human nutrition and its potential role in the prevention of various chronic diseases associated with oxidative stress. Consuming of foods rich in natural antioxidants, as well as processed foods enriched with them, provides the desired supply of antioxidants and helps in preventing diseases in which oxidative stress is a key pathogen (Hardy, 2000.). In accordance with the previous statement, plants like purple Echinacea would be particularly important and effective. Polyphenols are deliberately added to functional foods (Wyk and Wink, 2004; Buřičova et al., 2008; Sakač et al., 2005; Huang et al., 2010), and can also often be found naturally in foods in quantities that have active effect on human health. Purple Echinacea herb extracts could serve as antioxidants that can be added for prevention of auto-oxidation spoilage of food, but also as functional ingredients that can act antimutagenic and anticarcinogenic and thus reduce the potential damage of the organism from the mycotoxins that often contaminate food products.

Table 2. Median values of sensory parameters for peanut treated with pink echinacea extract

PARAMETERS	SAMPLES	KK	EK1	EK2	EK3
Appearance	color	9±1.5	8.3±1.6	8.2±1.6	8.5±1.6
Aroma	fried peanuts	7.8±2.2	6.0±2.3	5.9 ^a ±2.7	5.2 ^a ±2.7
	moist chalk	0±0	0.1±0.3	0.3±0.8	0.9 ^a ±1.5
	oxidised	0±0	0.3±0.7	0.3±0.8	0.2±0.6
	burnt	0±0	0.3±0.8	0.2±0.6	0.3±0.7
	atypical aroma	0±0	0.5±0.5	1.0 ^a ±1.1	1.7 ^a ±1.5
Taste	sweet	2.3±1.7	2.7±1.6	2.1±1.8	1.8 ^a ±1.4
	bitter	1.0±2.1	1.2±2.0	1.0±1.8	0.8±1.7
	salty	6.2±2.1	3.3 ^a ±2.5	2.9 ^a ±2.3	2.4 ^a ±2.1
	sour	0±0	0.1±0.3	0.0±0.2	0±0
Texture	hardness	5.5±1.0	5.6±1.2	5.7±1.7	5.0±1.9
Odor	typical odor	7.4±1.9	6.1±2.0	6±2.0	6±1.9
	atypical odor	0±0	0.1±0.5	0.1±0.5	0.1±0.5

KK: peanut control sample; EK1: pink echinacea extract treated peanut (0.1 mg/mL); EK2: pink echinacea extract treated peanut (0.5 mg/mL); EK3: pink echinacea extract treated peanut (1.0 mg/mL); ^a – the value is significantly different from control KK (p<0.05).



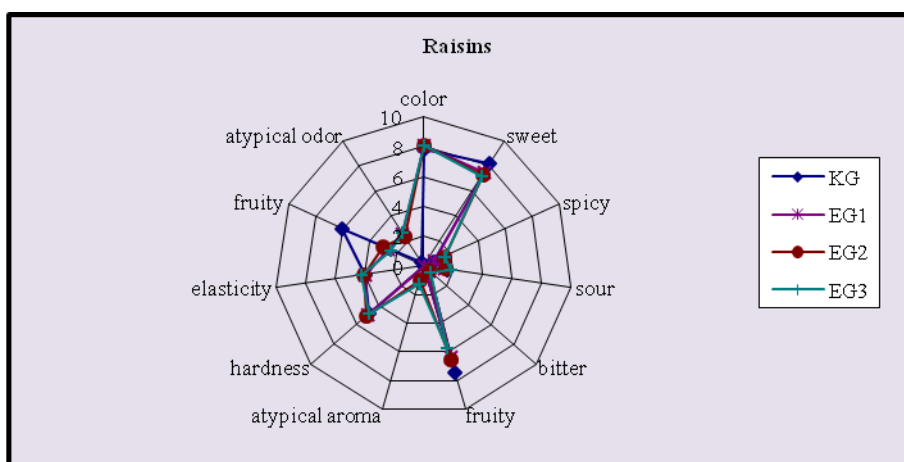
KK: peanut control sample; EK1: pink echinacea extract treated peanut (0.1 mg/mL); EK2: pink echinacea extract treated peanut (0.5 mg/mL); EK3: pink echinacea extract treated peanut (1.0 mg/mL). ^a – the value is significantly different from control KK ($p < 0.05$).

Fig. 2. Sensory profile of peanut samples treated with purple Echinacea extract

Table 3. Median values of sensory parameters for raisins treated with pink echinacea extract

PARAMETERS	SAMPLES	KG	EG1	EG2	EG3
Appearance	color	7.8±1.5	8.1±1.6	8.1±1.8	8.1±1.8
Taste	sweet	8.2±1.4	7.5±1.3	7.3±1.4	7.2±1.8
	spicy	0.7±1.5	0.8±1.4	1.5 ^a ±2.3	1.5 ^a ±2.4
	sour	1.6±1.7	1.4±1.7	1.5±1.7	1.8±1.8
	bitter	0.5±1.4	0.5±1.4	0.5±1.4	0.6±1.4
Aroma	fruity	7.4±2.1	6.4±2.6	6.5±2.6	5.7±3.1
	atypical aroma	0.0±0.2	0.0±0.7	1 ^a ±1.8	1.3 ^a ±2.1
Texture	hardness	4.9±0.9	5.0±1.1	5.1±1.1	4.91±1.3
	elasticity	4.0±1.4	4±1.5	4.1±1.5	4.2±1.8
Odor	fruity	6±2.2	2.6 ^a ±2.8	3 ^a ±3.0	2.4 ^a ±2.9
	atypical odor	0.3±0.6	2.6 ^a ±3.1	2.4 ^a ±3.2	2.7 ^a ±3.3

KG- raisin control sample; EG1: raisins treated with pink echinacea extract (0.1 mg/mL); EG2: raisins treated with pink echinacea extract (0.5 mg/mL); EG3: raisins treated with pink echinacea extract (1.0 mg/mL); ^a – the value is significantly different from control KG ($p < 0.05$).



KG- raisin control sample; EG1: raisins treated with pink echinacea extract (0.1 mg/mL); EG2: raisins treated with pink echinacea extract (0.5 mg/mL); EG3: raisins treated with pink echinacea extract (1.0 mg/mL). ^a – the value is significantly different from control KG ($p < 0.05$).

Fig. 3. Sensory profile of raisin samples treated with purple Echinacea extract

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